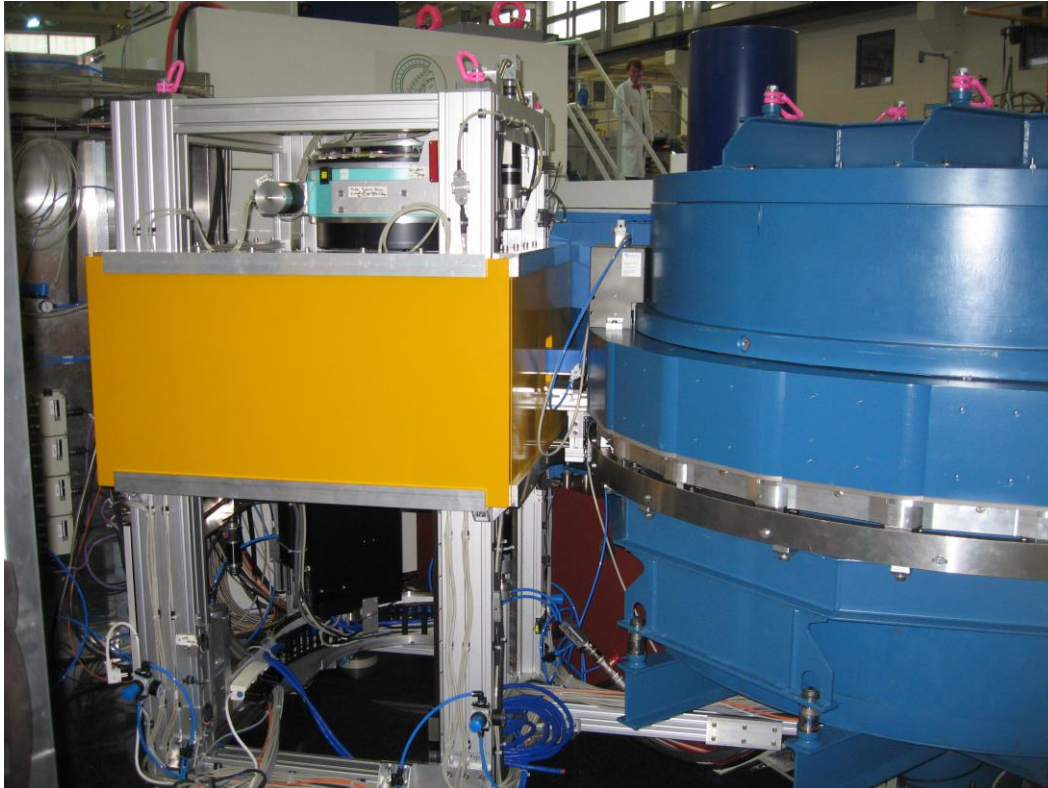


Combined neutron and light scattering analysis on the crystallization of the model protein Lysozyme

28.05.2015

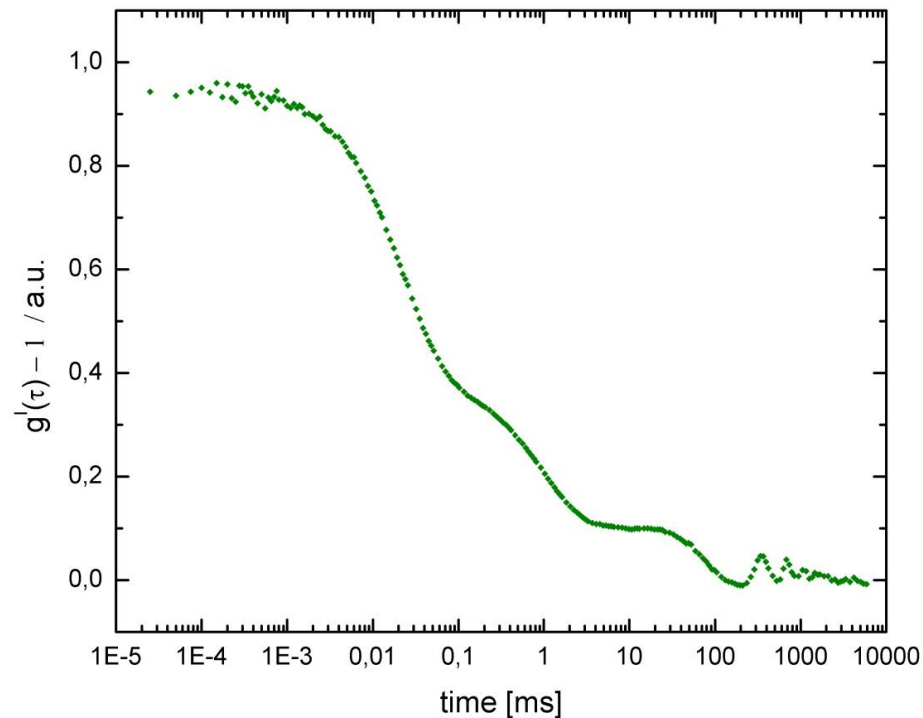
Tobias E. Schrader



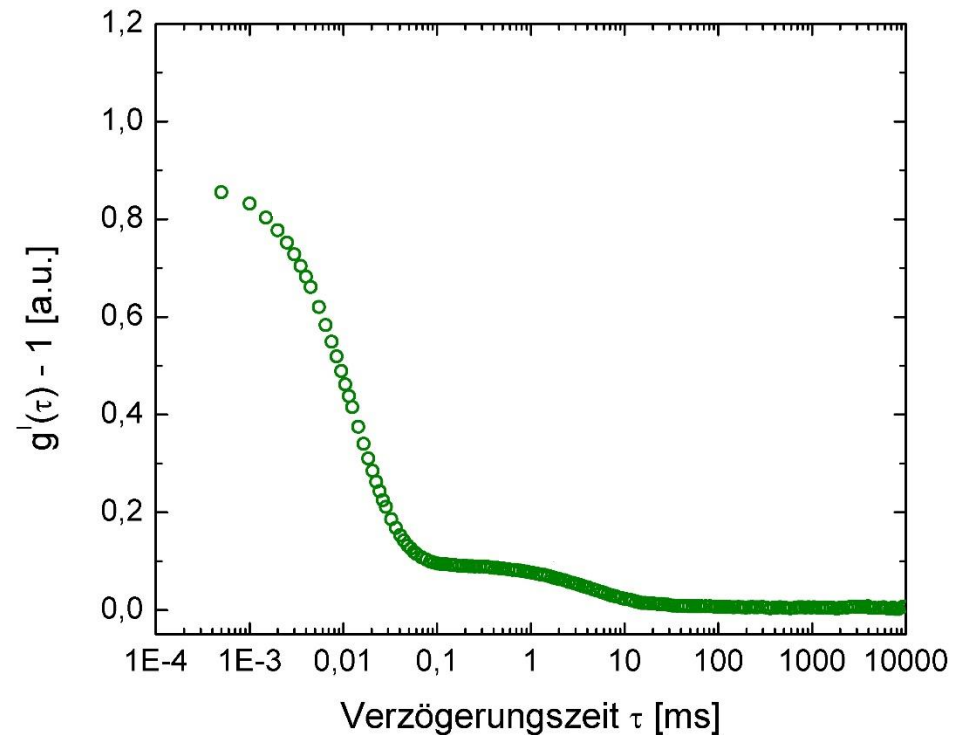
Necessary crystal size:
At least 0.5 mm^3

- Deeper understanding of the underlying crystallization mechanism is required

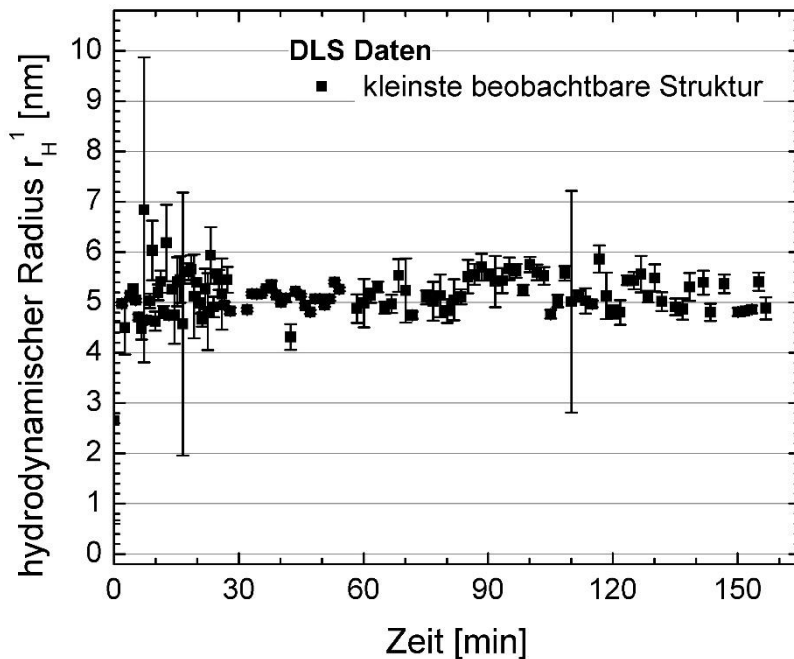
$T = 294,5 \text{ K}$



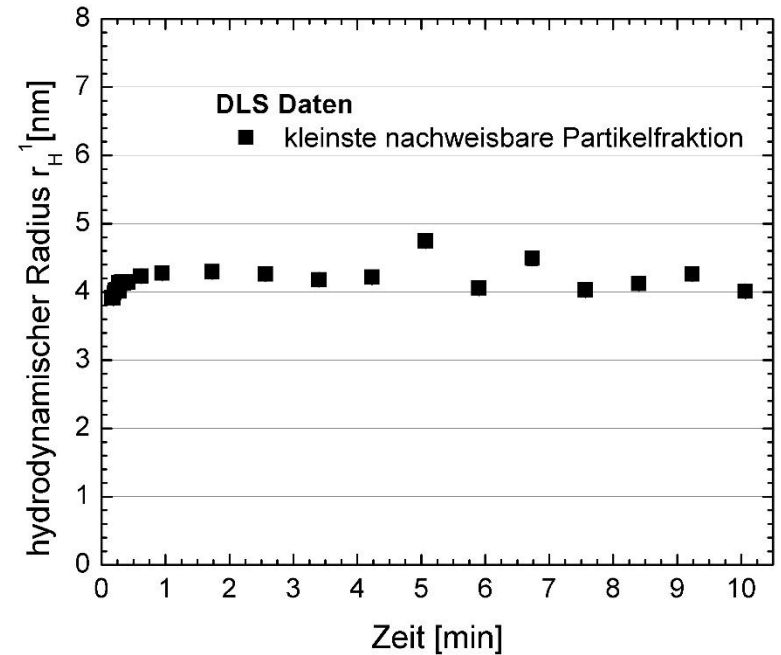
$T = 298 \text{ K}$



T= 294,5 K

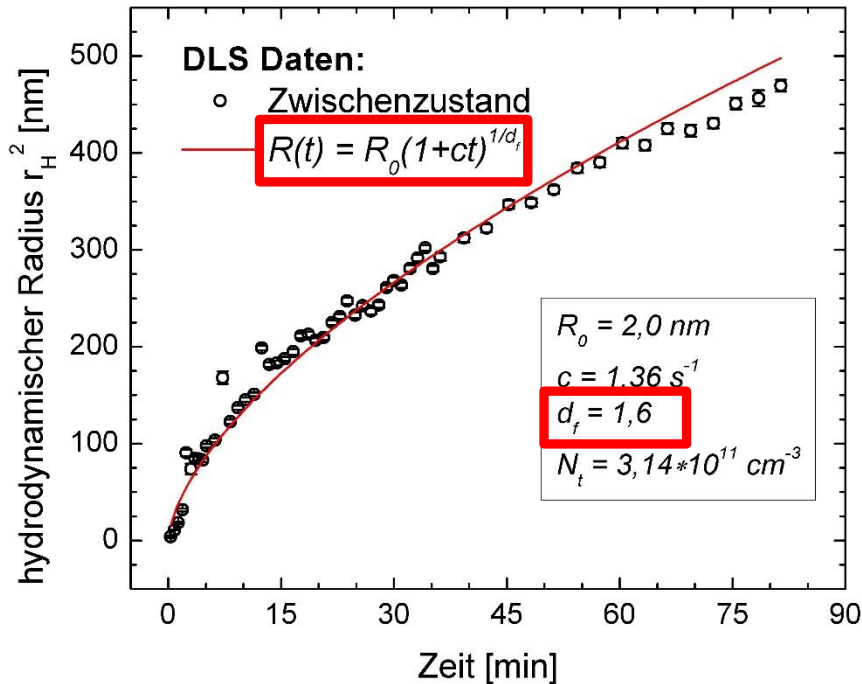


T= 298 K



➤ Constant radius of the dimer fraction in both cases

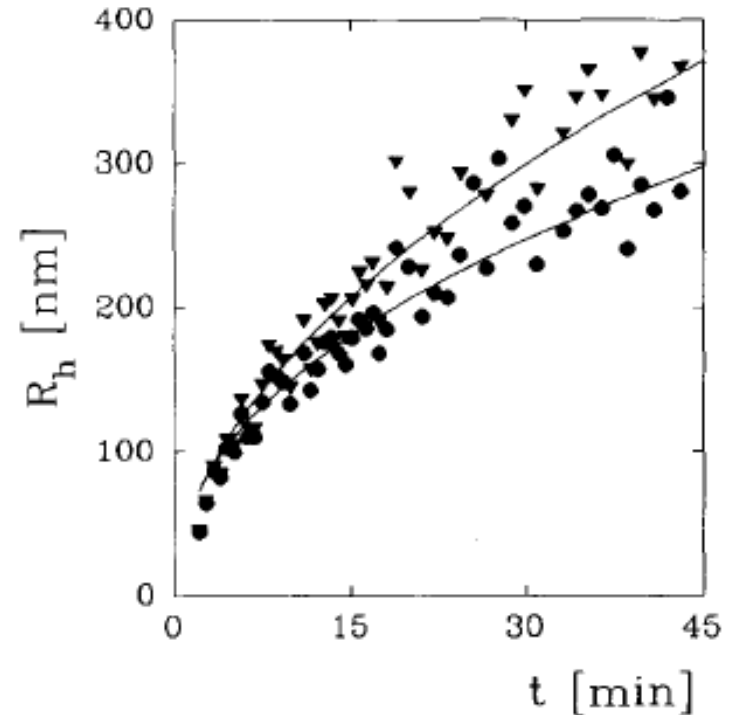
T = 294,5 K



DLS with 60mg/ml Lysozyme mixed with 6wt% in D₂O Puffer

pH 4.35; T = 294.5 K; scattering angle 174°

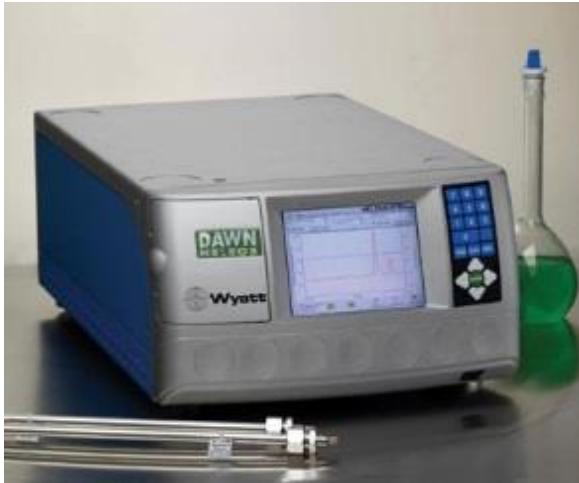
Y. Georgalis, A. Zouni, W. Eberstein, W. Saenger, Crystal Growth 126, 245-260



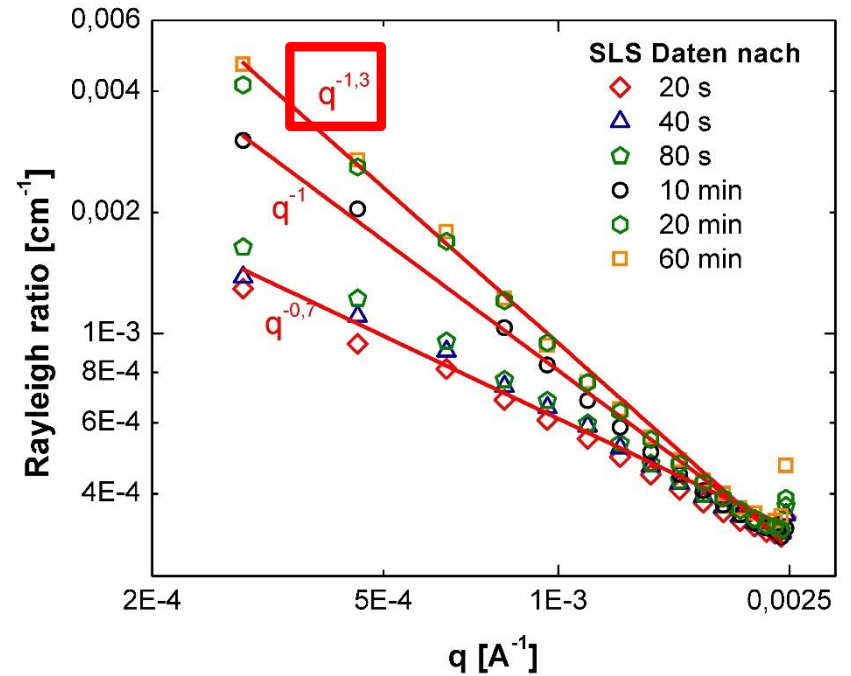
DLS with 61.3 mg/ml Lysozyme mixed with 7.2wt% NaCl in H₂O Puffer

pH 4.2; T = 293 K; scattering angle 20°

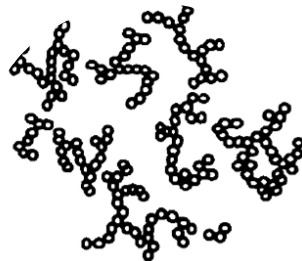
T= 294.5 K



$d_f = 1,3$

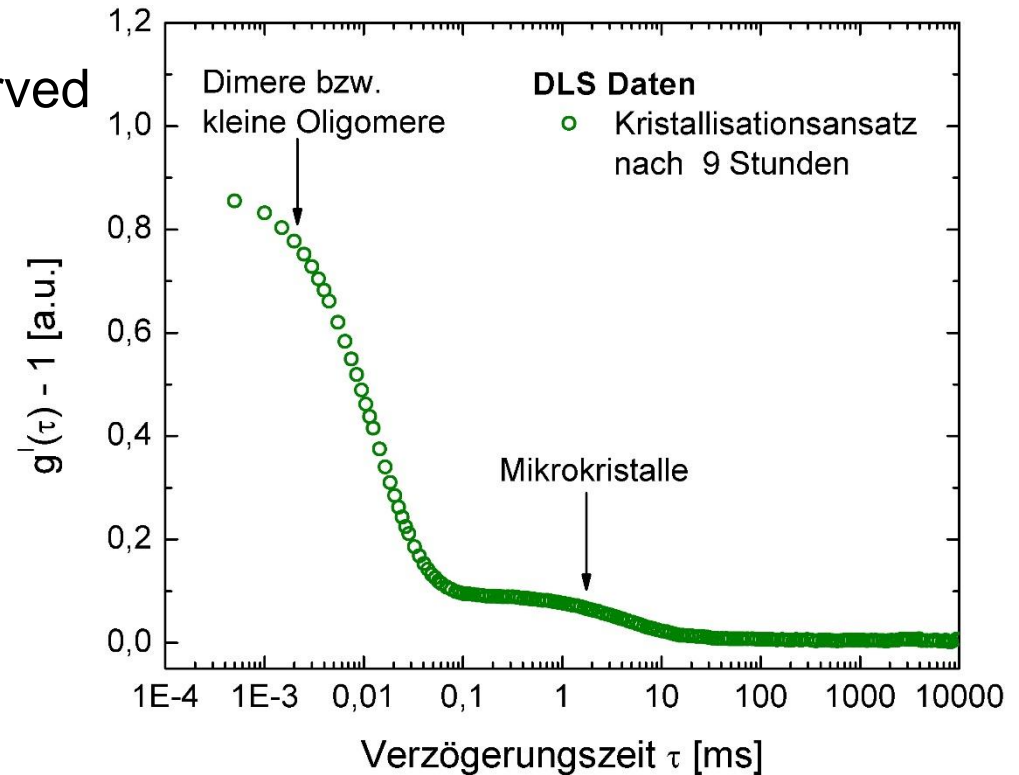
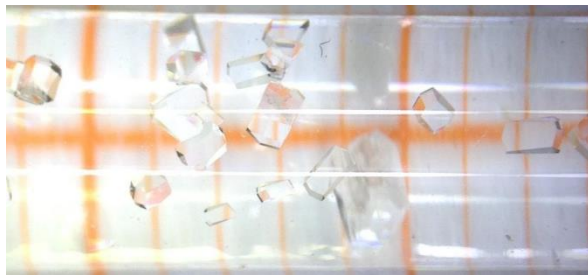


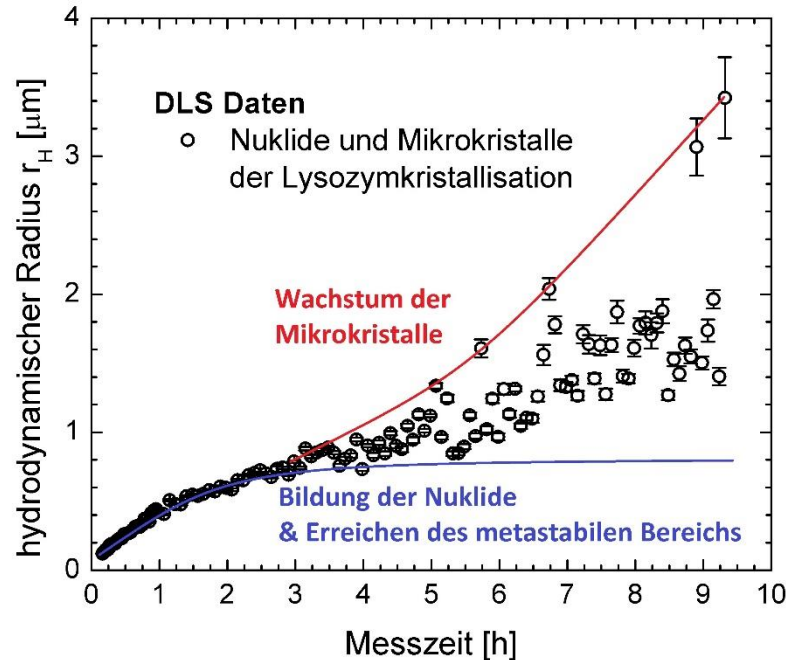
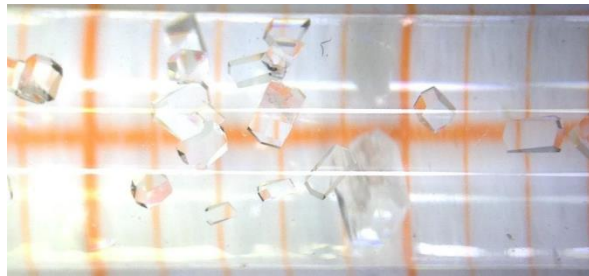
Fractals form!



T= 298 K

- No third particle fraction observed
- Crystals grow larger in size as at 294.5 K





T = 298 K

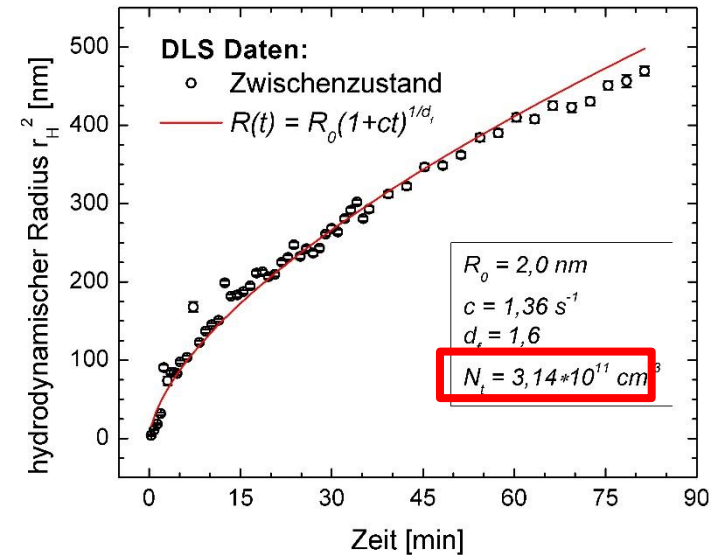
- In the beginning we have two particle fractions
- After three hours the sample is not ergodic any more: Large size fluctuations in the larger size fraction is observed
- Interpretation: Small crystals diffuse through the observation volume

Small angle scattering signal can be calculated using a model fit of the DLS data

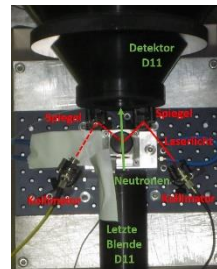
Volume of the crystal nucleus

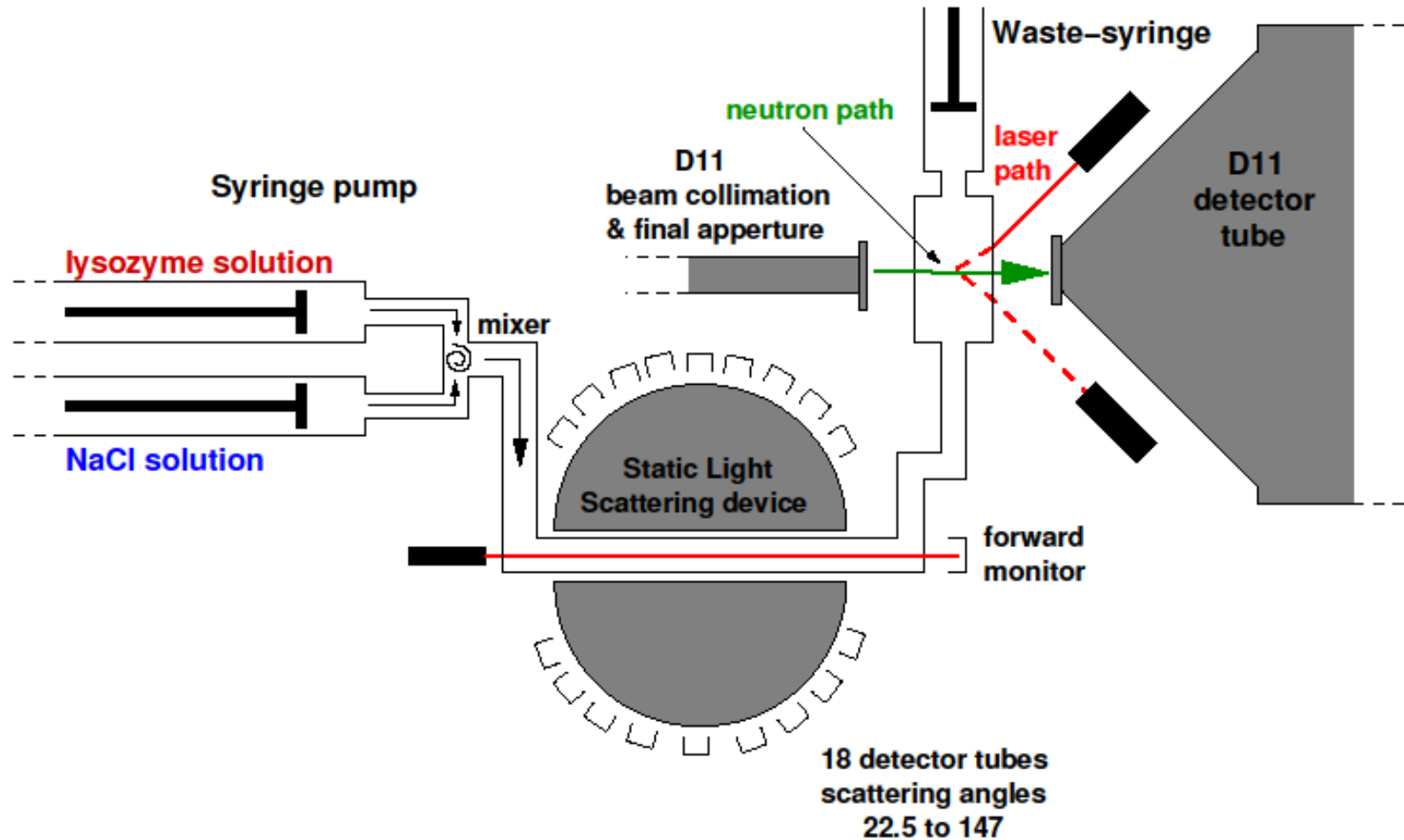
$$\frac{d\Sigma}{d\Omega}(q) = \frac{N_t}{V} * (\Delta\rho)^2 * V_p^2$$

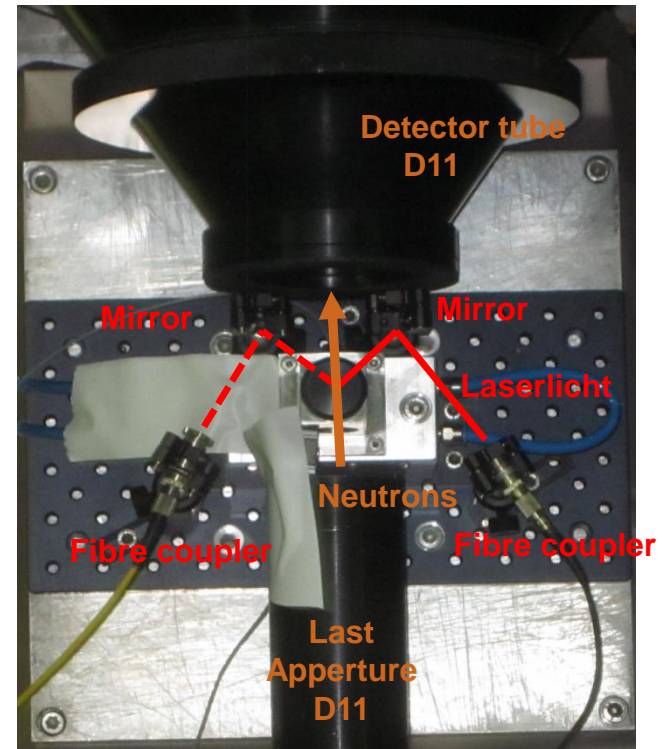
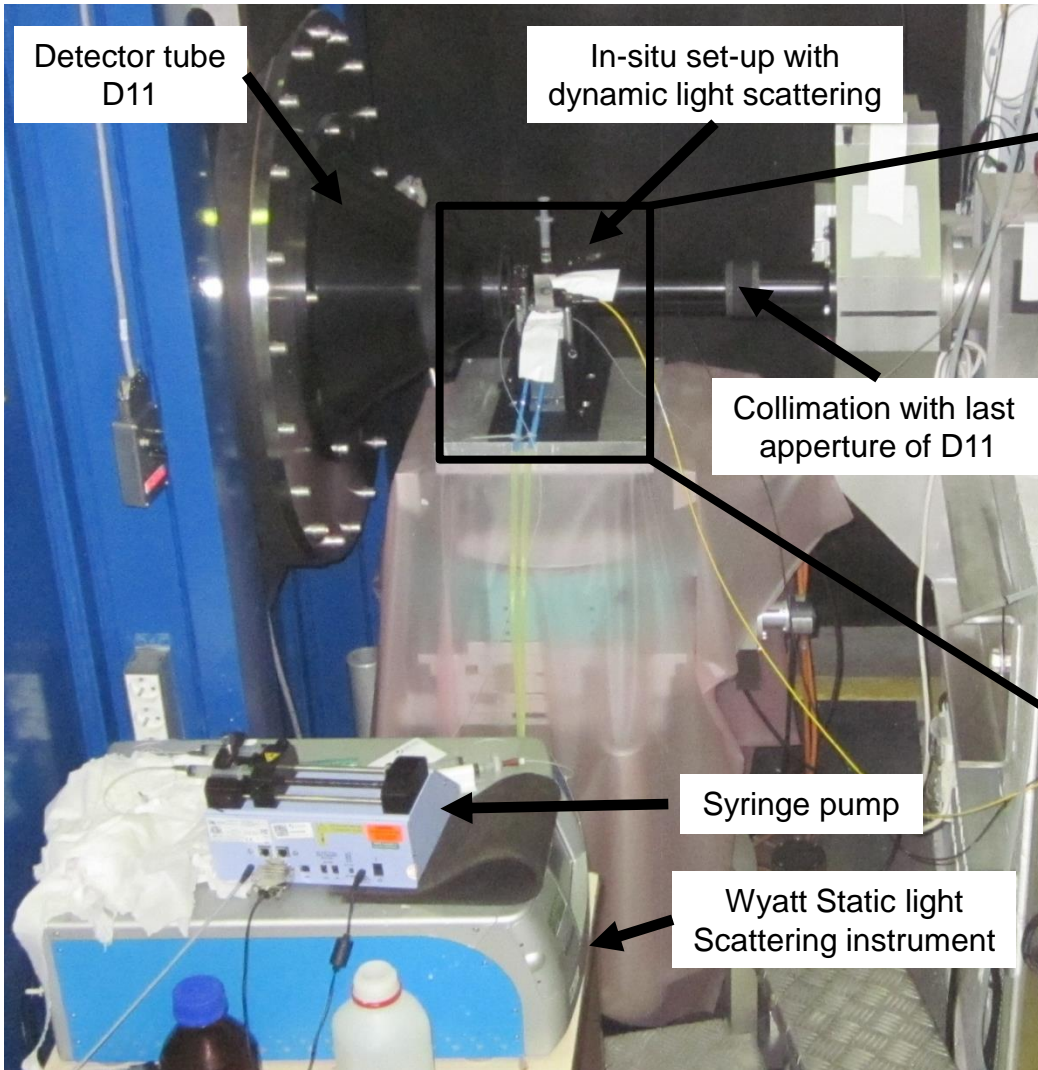
Scattering contrast of lysozyme



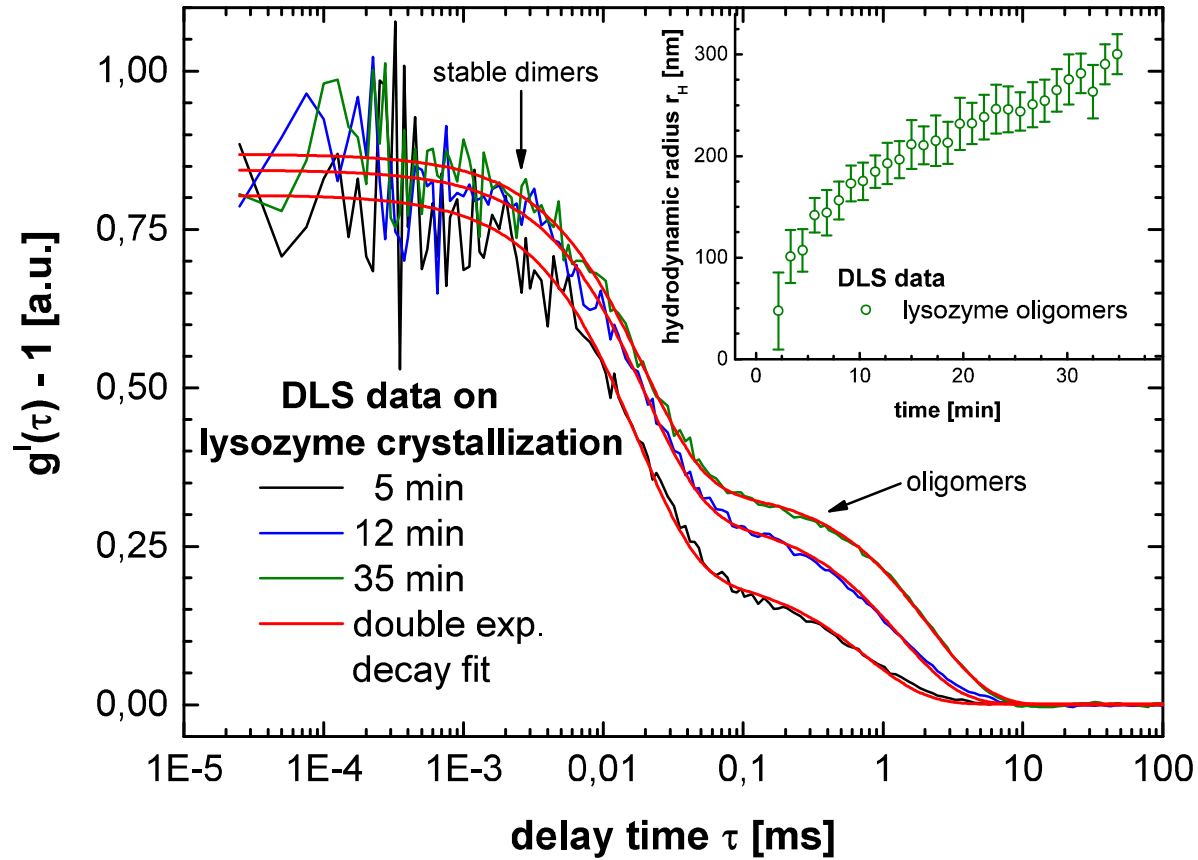
Time resolved structural information on the Lysozyme crystallization: In-situ **DLS** and quasi-in-situ **SLS** together with **Small angle neutron scattering (SANS)**



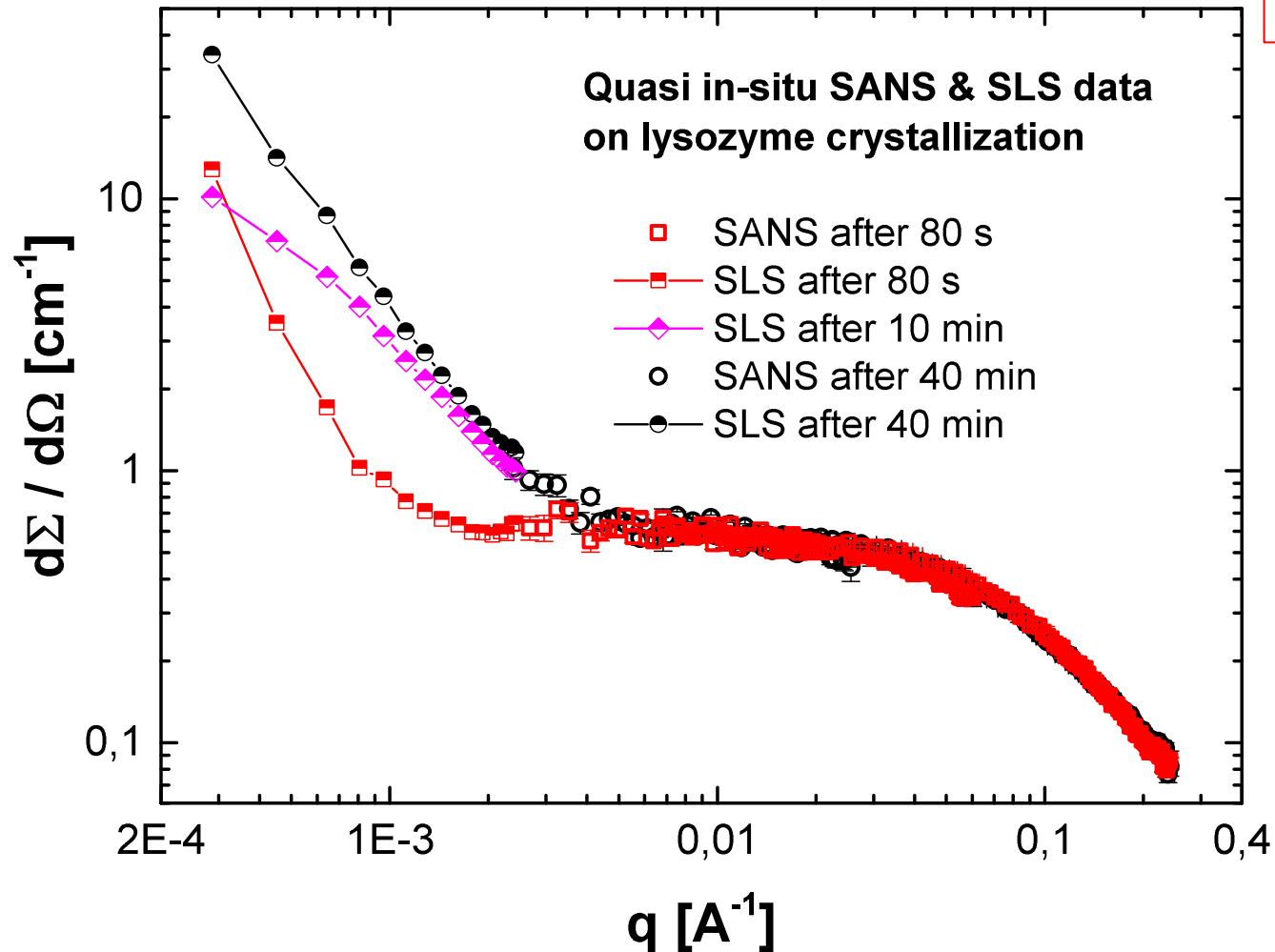


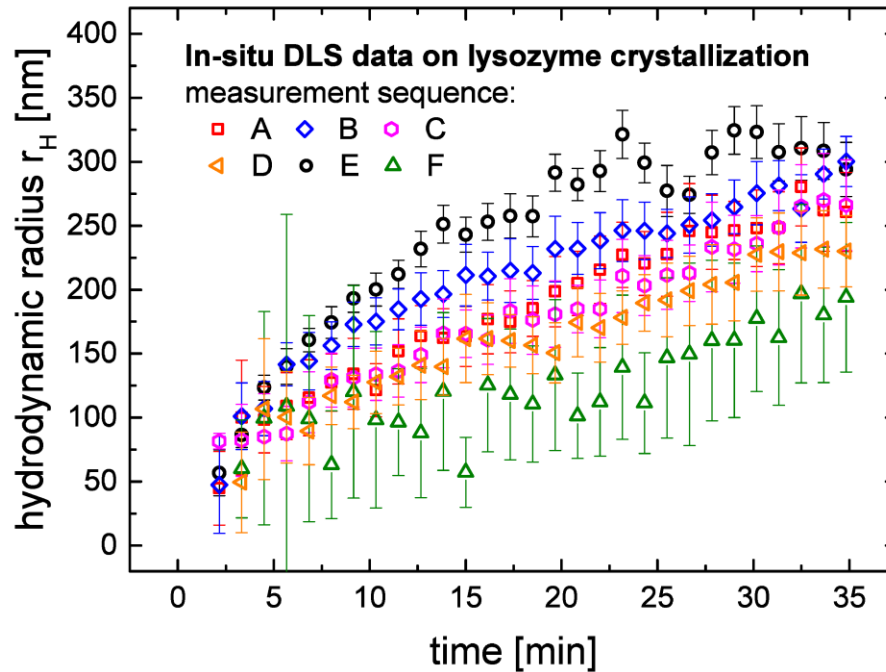


T = 298 K



T = 298 K



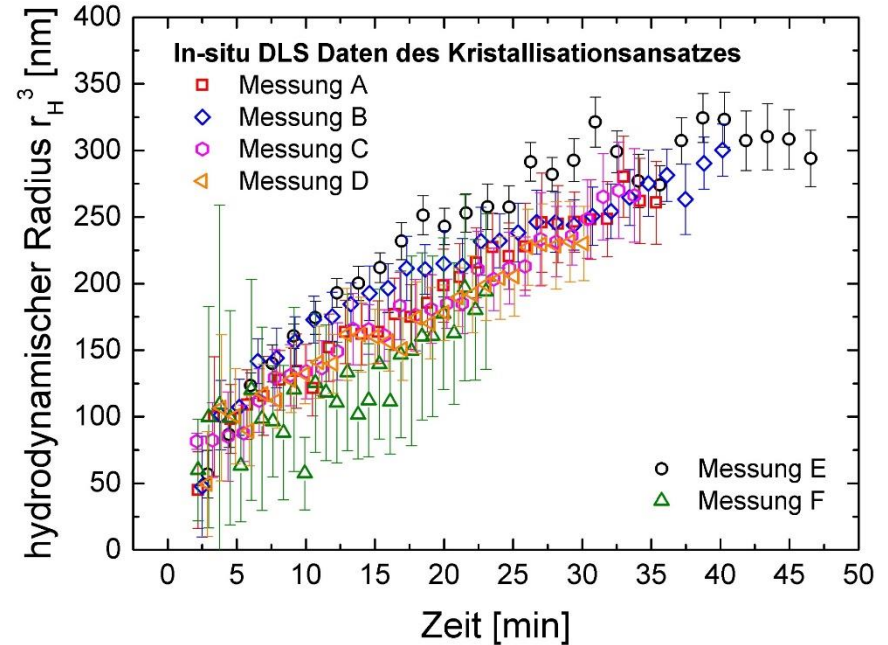
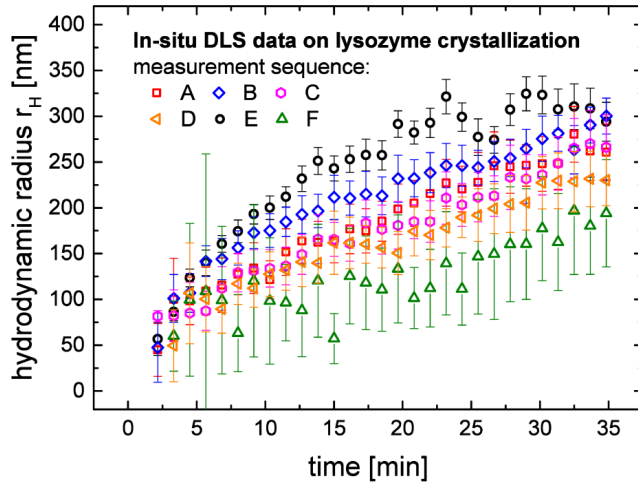


Differences in the speed of the Crystallisation process:

- Possible reasons are fluctuations of the temperature in the vicinity of the sample cell

➤ Scaling factor necessary to account for the differences

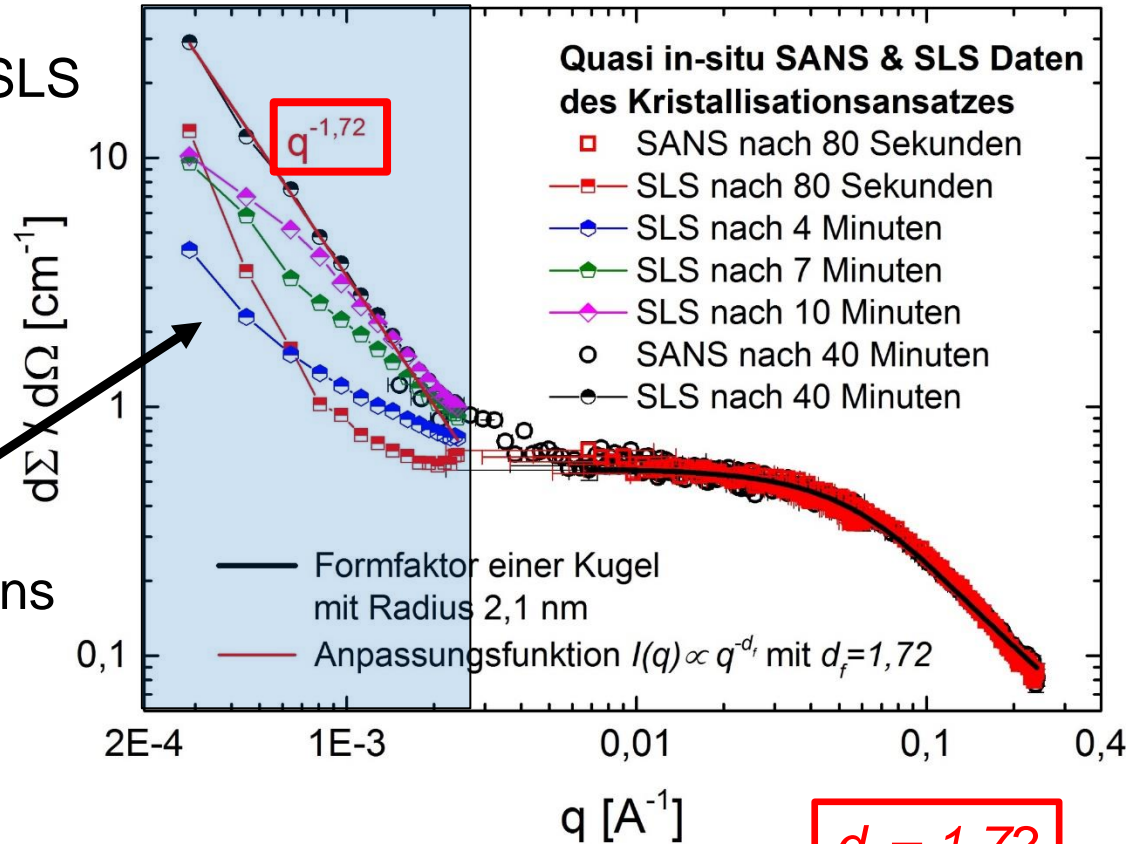
T = 298 K



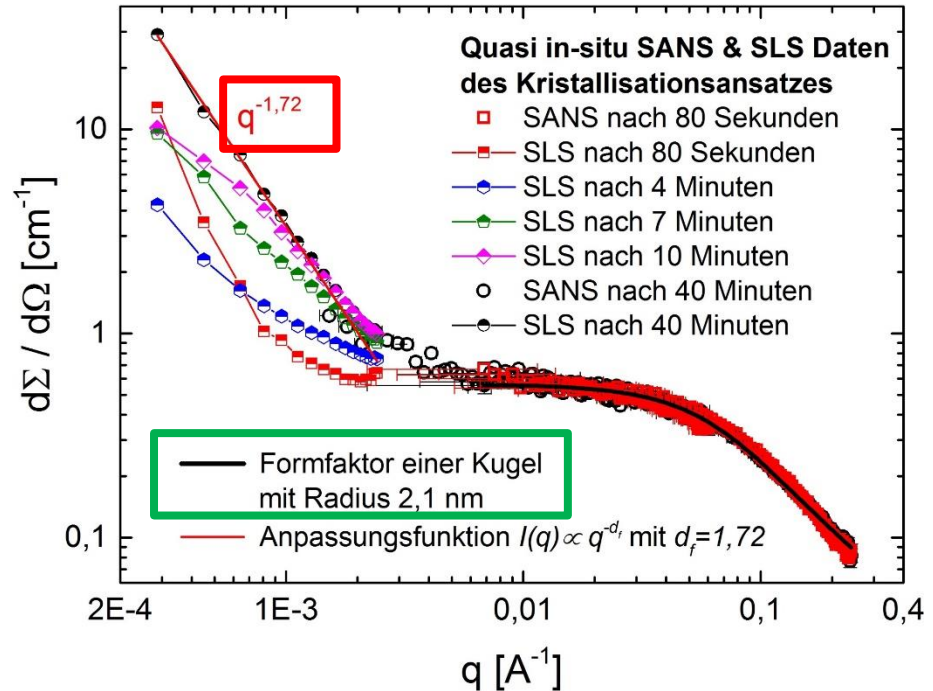
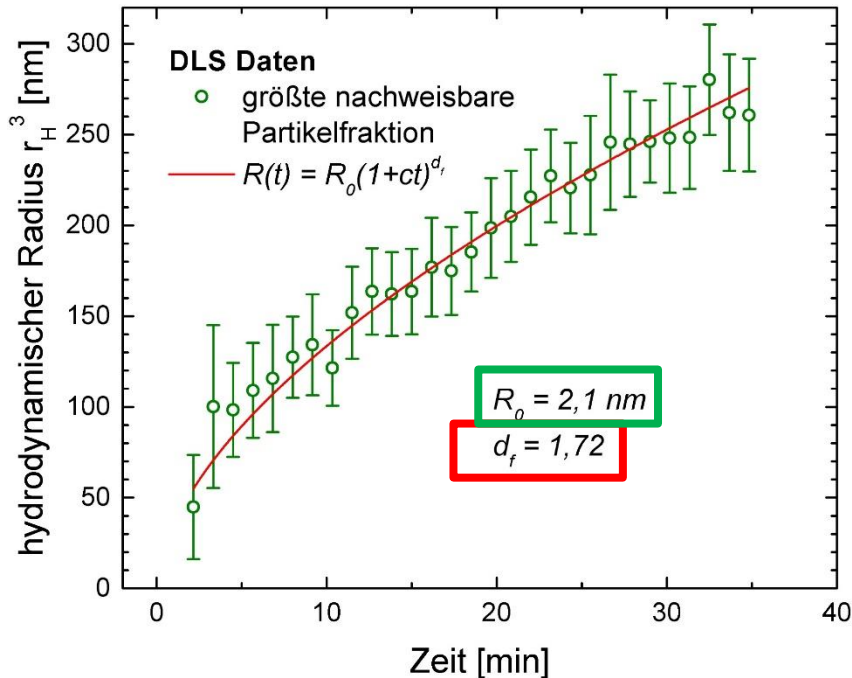
- A scaling factor can be determined to correct for tiny differences in crystallisation speed

T = 298 K

- Extended q-range due to SLS
- Time evolution of the structure of the lysozyme nuclei can be followed
- Change of fractal dimensions observed



T = 298 K

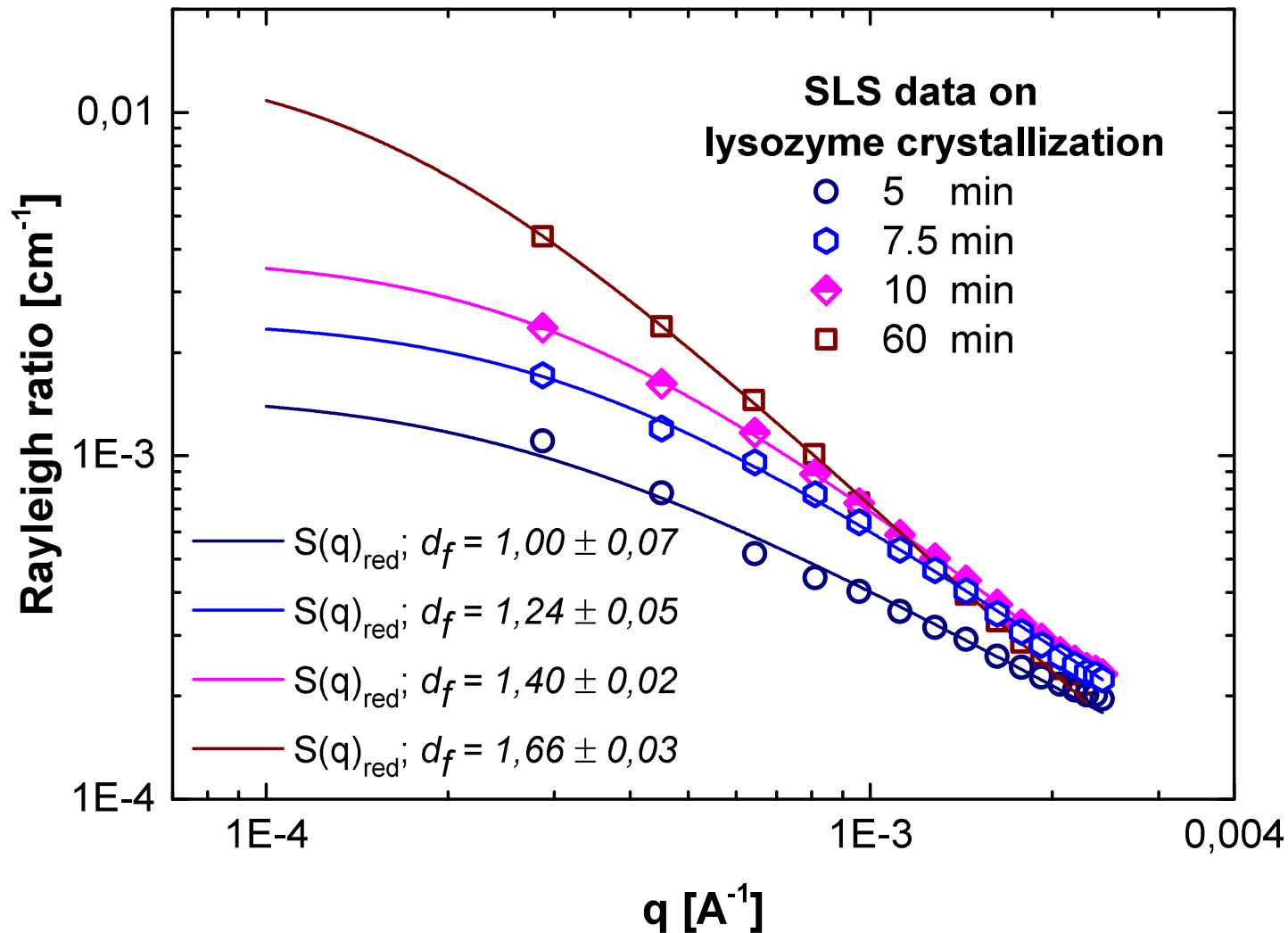


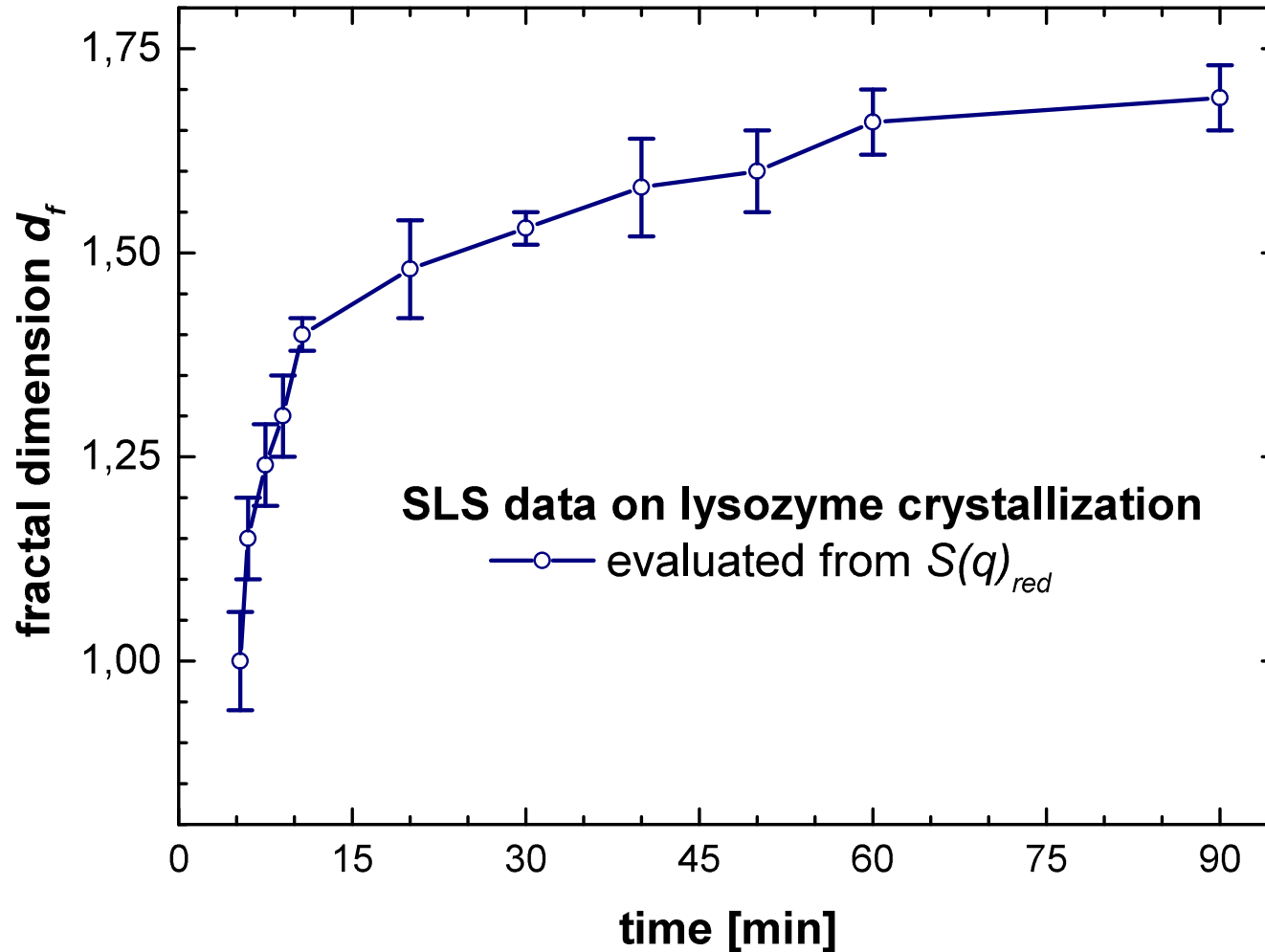
- Agreement of fractal dimension at 40 min. d_f
- Fixed parameter R_0 from SANS used for the model fit of the DLS data
- Verification of the diffusion limited aggregation model

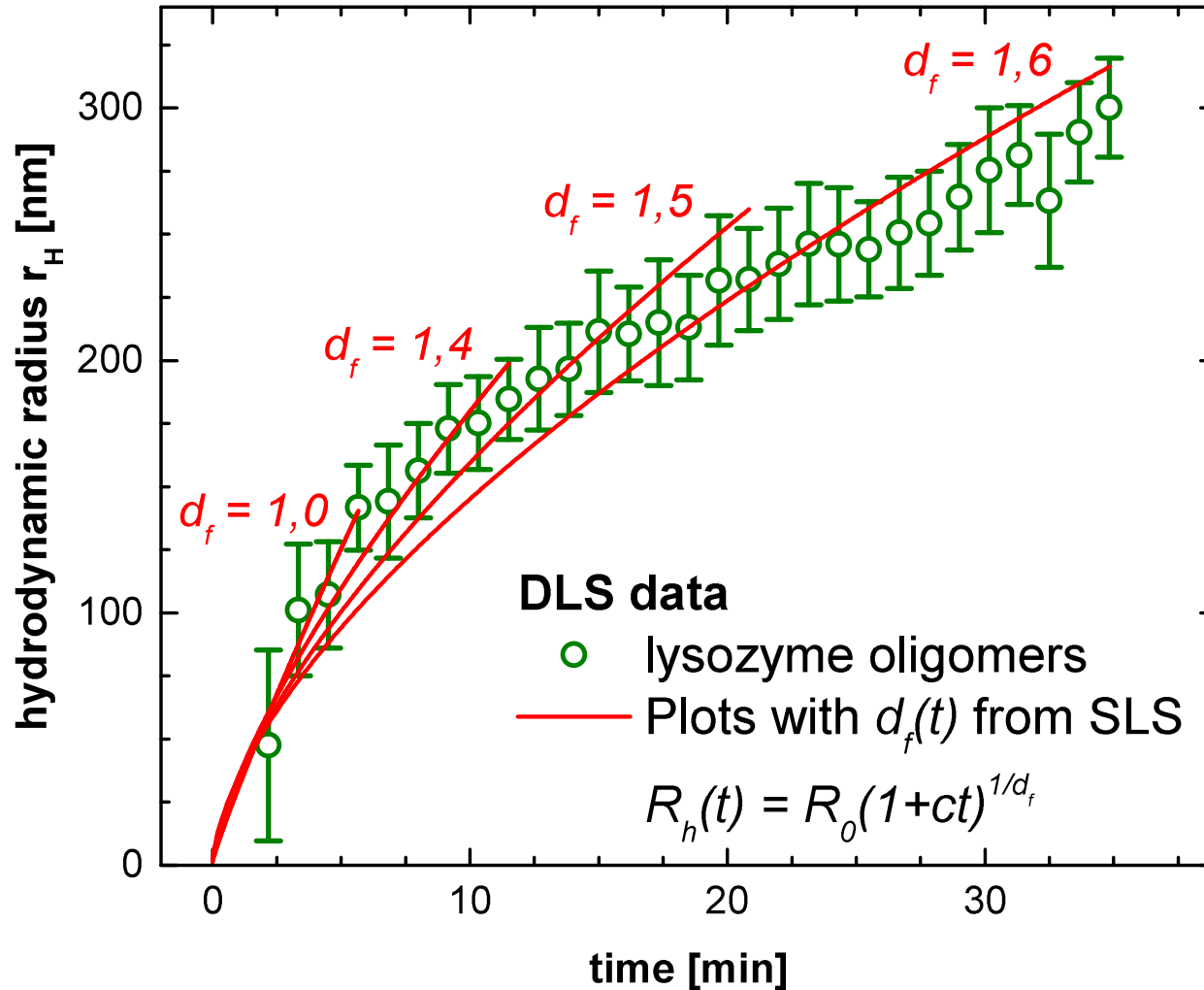
$d_f = 1,72$

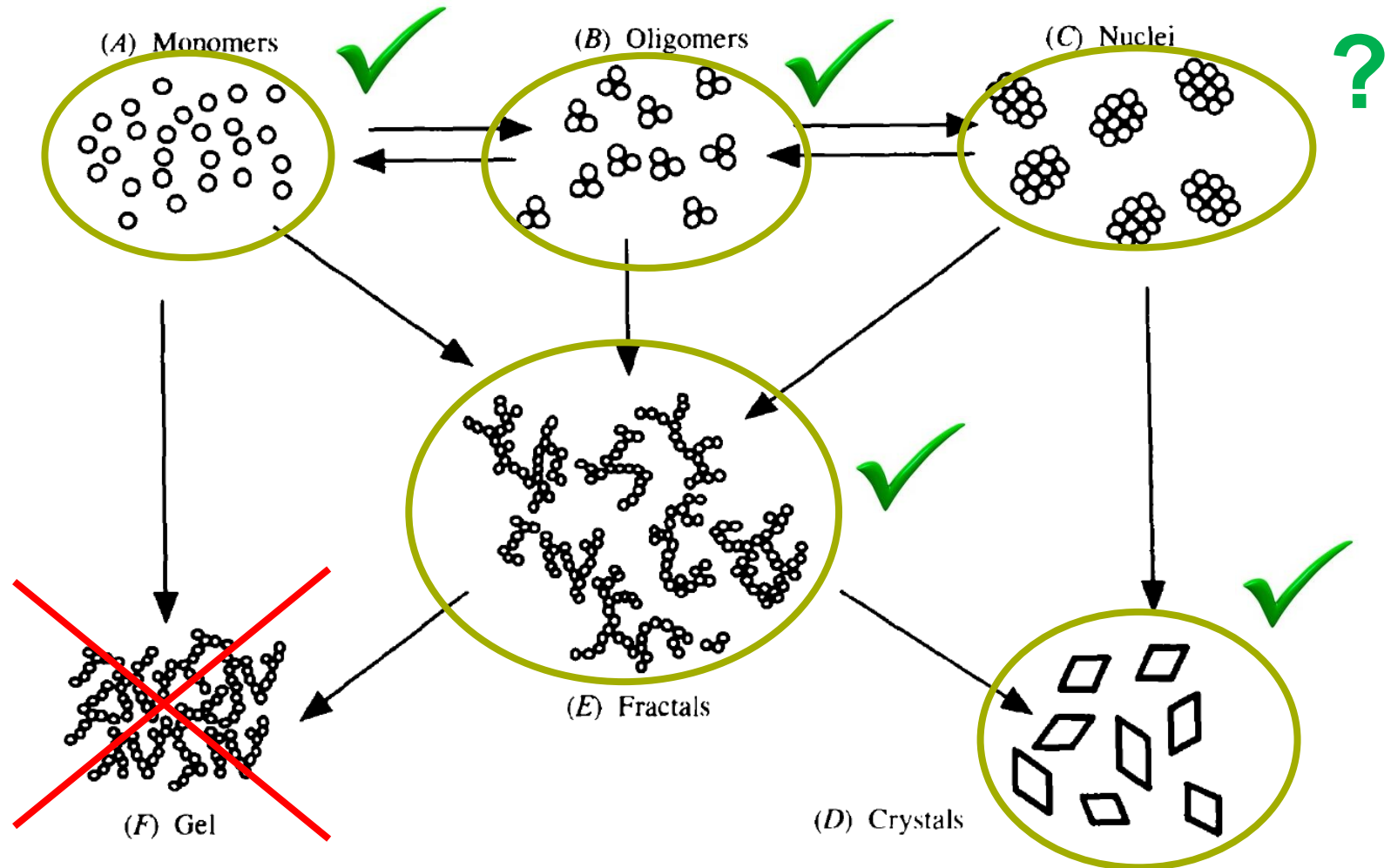
$T = 298 \text{ K}$

Just the SLS data is needed for fitting the fractal dimension

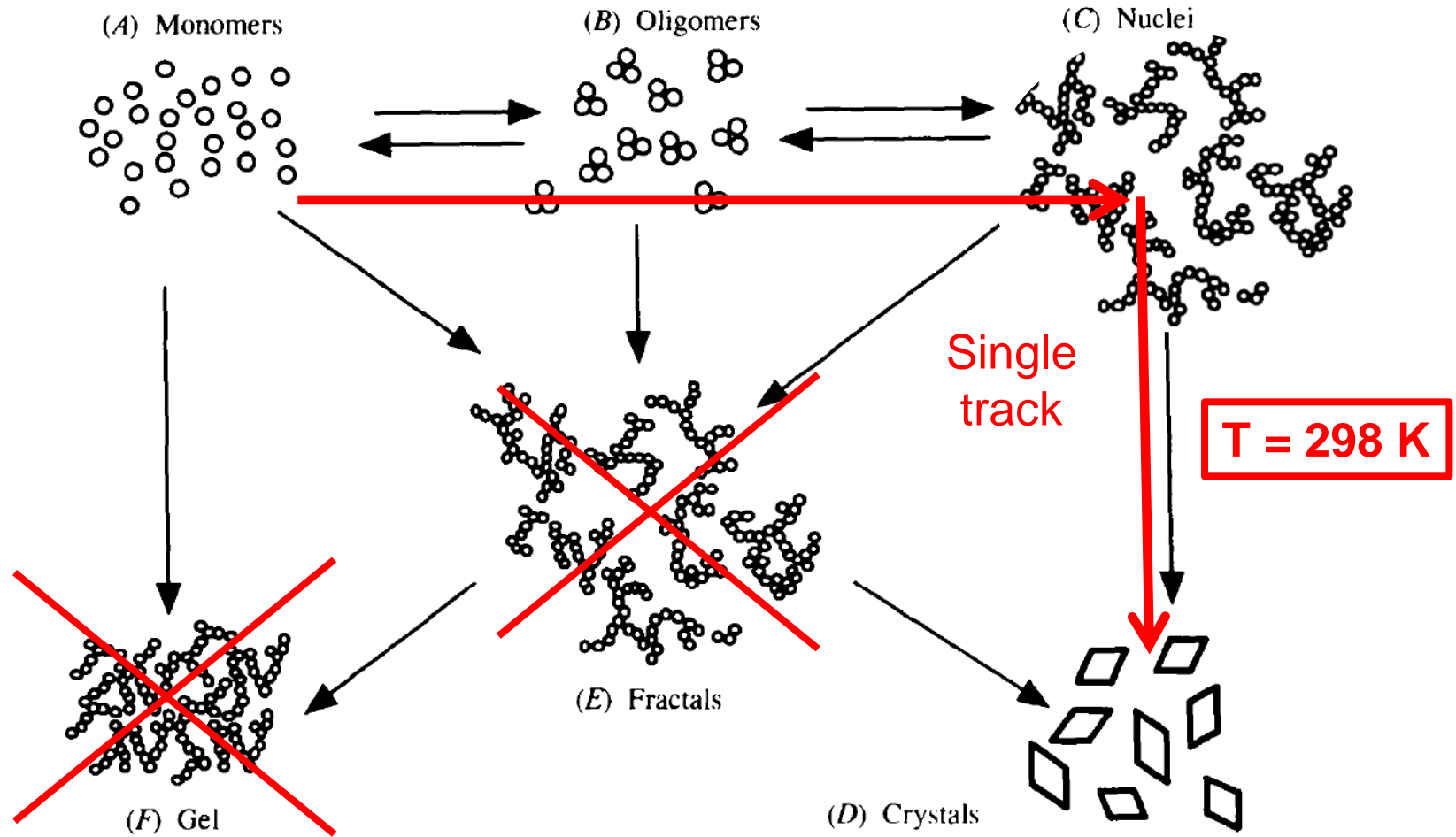




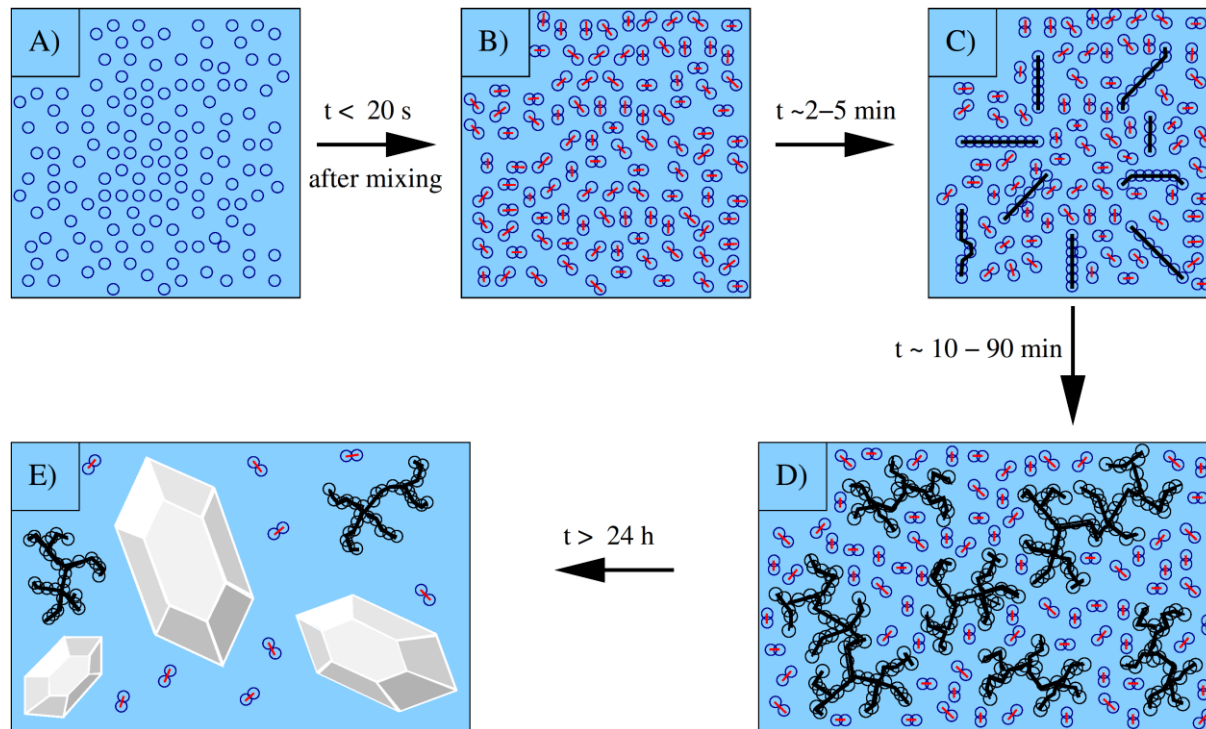




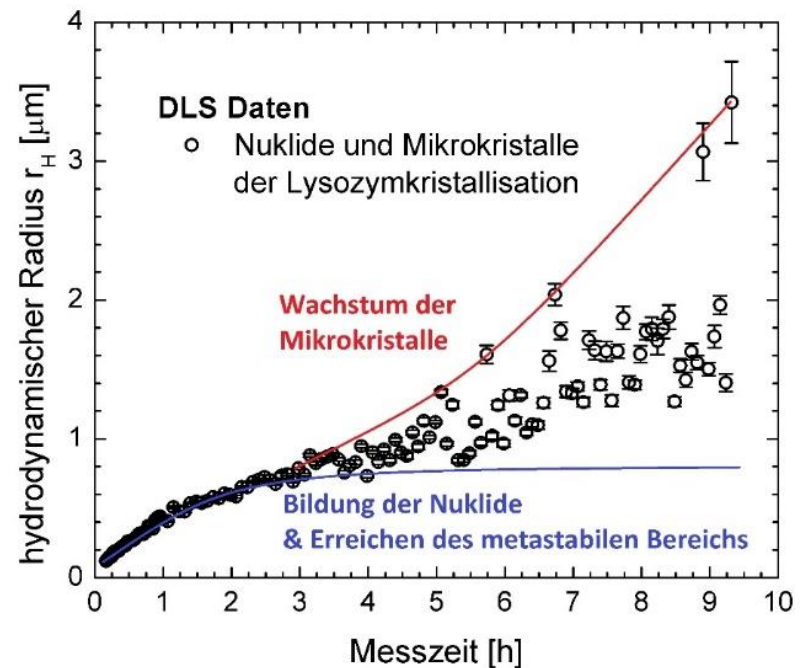
Y. Georgalis, P. Umbach, J. Raptis and Wolfram Saenger, Acta Cryst. 53 (1997) 703-712



Y. Georgalis, P. Umbach, J. Raptis and Wolfram Saenger, Acta Cryst. 53 (1997) 703-712



- Lysozym dimers/ small Oligomers
 - Size constant in time
 - Concentration decreases (consumption due to crystal growth)
- Lysozyme oligomers
 - Fractal Structure
 - Involved in crystal growth
 - Are not present at T=298 K
- Crystals
 - Growth at surfaces
 - Nucleation observed at T = 298 K
 - At the beginning: Fractal dimension with changing exponent



Differences in previous observations on the number of particle sizes resolved

- Temperature is the key parameter for different number of particle sizes observed
- The chosen method of observation also makes a difference

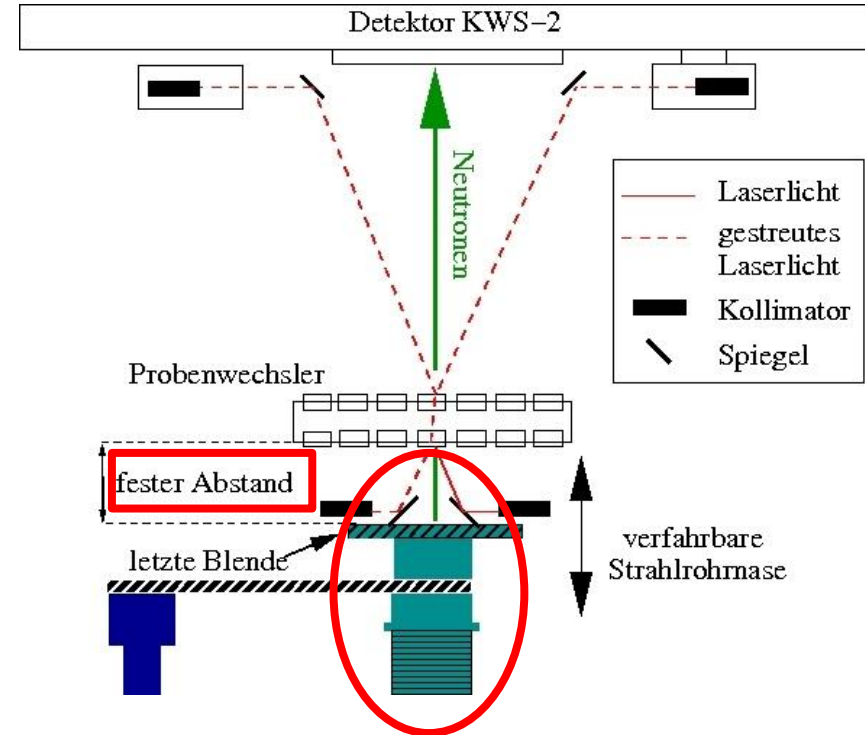
Successful observation of the nucleation phase with structural information

- Analysis of the speed of the nucleation process
- Not only size but also structural information gained

First successful and necessary application of the in-situ light scattering method

- With enlarged q-range
- Reproducibility checked

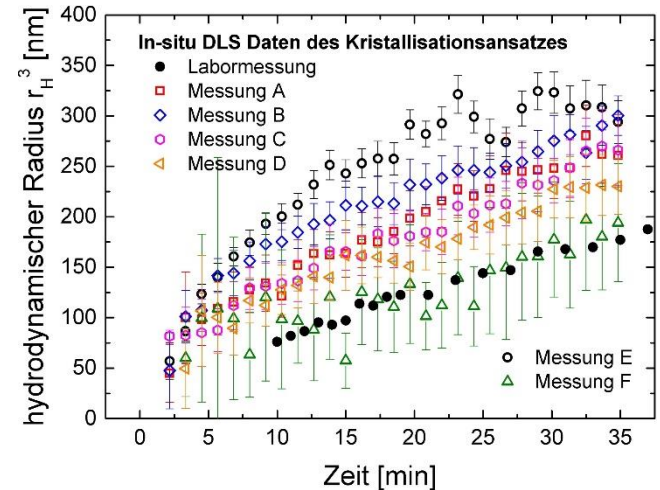
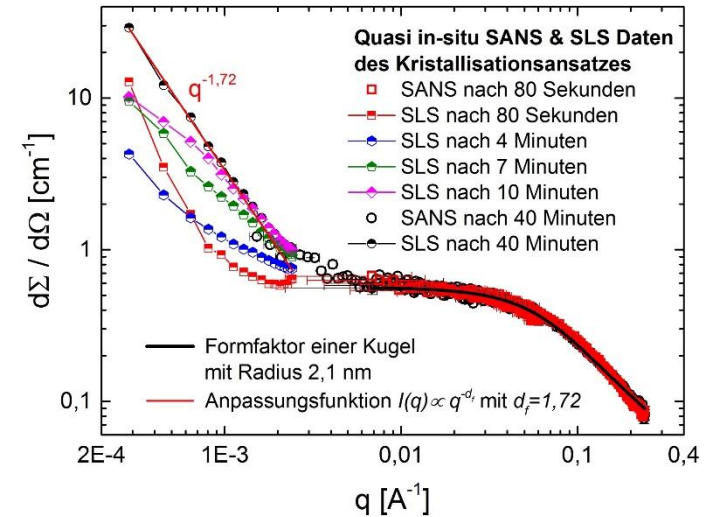
- In-situ DLS at KWS-2
 - Additional scattering angles
 - Moving final aperture



- In-situ DLS Versuche an KWS-2
 - Zusätzliche Streuwinkel
 - Verfahrbare Strahlrohrnase

- Protein crystallisation
 - Methods to increase the size of the crystals
 - Study of the nucleation process des

- Open questions
 - Informationen on the early times using a scaling factor to align the measurements, improved averaging of the neutron data
 - Kinetic model



Many thanks to... ... The D11 team:

- Joachim Wuttke
- Dieter Richter
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- Aurel Radulescu
- Jörg Stellbrink
- Ralf Schweins
- David Bowyer
- David Hess
- Emanuel Kenzinger

Thank you for your attention!